The Male Meiotic Phase in two Genera of Marsupials (Macropus and Petauroides).

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With Plates 12–14.

A very slight experience of cytological research is sufficient to impress the worker in this field with the different facilities for accurate research afforded by different organisms, and also with the importance of discovering the most favourable objects for such research. Consequently I have been making a cytological survey of such groups of Australian animals as seemed most likely to afford useful cytological material, paying at first particular attention to the Marsupials, for two reasons. The first is the well-known technical difficulties presented by the Eutherian mammals on account of the usually rather large number and tendency to clump of their chromosomes, and the second is that Jordan's work on the American opossum (1911) showed that in this marsupial the number of chromosomes is comparatively small. Jordan determined the number as seventeen (male), but Painter (1922) has raised it to twenty-two. Up to the present we have made in this laboratory a preliminary survey of some fourteen species of Marsupials, and this paper presents an account of the more important features of two of these which have been worked out in more detail.

At present the most interesting feature of this work is undoubtedly the determination of the conditions of the sex chromosomes, a problem which has always presented in mammals considerable elements of dubiety. Of primary importance is probably the confirmation of the occurrence of

Y-chromosomes in the Mammalia—first clearly established by Painter (1922) in Didelphys. In Macropus this element is very minute, and, though I had shown it in several figures, I failed to interpret it properly until we received Painter's paper, which arrived at a moment when Mr. Greenwood (whose results are to be published in this journal) was paying attention to a very minute chromatic body occurring in several other species of Marsupials at which he was working. It immediately became apparent that this body was the Y-chromosome. Comparison with his work, and with Painter's description of Didelphys, quickly established that the small body already observed in Macropus is also a Y-chromosome.

It is a pleasure to acknowledge my indebtedness to several people for assistance in obtaining this material. Dr. T. L. Bancroft, of Eidsvold, Queensland, has sent me in the last two years a great number of specially preserved testes of Marsupials, Monotremes, Ceratodus, and many other Australian animals. For living material utilized in the present paper I have to thank Dr. Colin MacKenzie, Director of the Australian Institute of Anatomical Research, and Mr. W. H. D. Le Souef, of the Zoological Gardens, Melbourne.

Material and Methods.

The two species dealt with in the present communication are Macropus ualabatus and Petauroides volans. These genera belong respectively to the Diprotodont families Macropodidae and Phalangeridae.

The material was mostly preserved in Flemming, Bouin, and Allen's modification of Bouin (1913, 1915). I have found the latter method excellent, especially when followed, as Allen recommends, by anilin and bergamot oils in place of the higher alcohols and xylol. For general purposes I have found this method the best I have yet tried for mammals.

The standard stain used was Heidenhain's iron haematoxylin, though safranin, methyl green, and acid fuchsin, and others, were used as controls.

As in former work, I have found thick sections much more

valuable than the thin ones usually used by cytologists. Most of the work has been done with sections of 15–20 μ in thickness, mounted between two coverslips instead of in the usual way between a slide and a coverslip. This allows any nucleus to be examined from both sides. For convenience of examination the lower coverslip is temporarily attached to a microscope slide by a drop of immersion oil.

A. Macropus.

Number of Chromosomes.—This animal is remarkable among mammals for its small number of chromosomes. The chromosome formula is of the type first clearly established for mammals in the case of Didelphys (Painter, 1922), the males being of the XY type. The diploid chromosome number in the male is 10+XY, or twelve, and in the meiotic division there are five autosome bivalents and the XY bivalent. This very small number allows of great certainty in counting the chromosomes, Macropus being obviously far more favourable for this purpose than any other known mammalian genus.

Although counts of the meiotic chromosomes leave no doubt that the number is as just stated, it is only rarely that there are twelve separate chromosomes in the spermatogonial mitoses. As a rule there are quite indubitably only eleven (Pl. 12, fig. 2), of which one, usually occupying the centre of the ring, is very minute. This is the Y-chromosome. In a small percentage of cases, however, there are equally plainly twelve (Pl. 12, fig. 3), the extra one being smaller than any of the others except Y. This is obviously the X-chromosome, for the meiotic phase shows that X is much smaller than the autosomes. In the 11-chromosome spermatogonial mitoses X is presumably attached to one of the autosomes, though I have not been able to identify with certainty the chromosome to which it is joined. Owing to its small size it would not add much to the length of one of the longer chromosomes. I have frequently found a constriction near the end of one of the longer chromosomes, but in view of the widespread tendency of chromosomes to develop such constrictions it would be unjustifi-

able to assume that this represents the point of attachment of the X-chromosome.

As will be described in more detail below, similar conditions are found in the meiotic division. The XY bivalent is possibly sometimes independent, but more often it is attached to one of the autosomes. In this phase, however, the XY is easily identifiable even when attached to an autosome.

In the fact of its usual attachment to an autosome but occasional independence, both in spermatogonial and meiotic mitoses, the X-chromosome in Macropus resembles that of Ascaris megalocephala (Edwards, 1910). In many male Orthoptera also the X-chromosome is temporarily or permanently united to an autosome (McClung, 1905; Wilson, 1911).

In the female (Graafian follicle cells) the small Y-chromosome, so characteristic of the spermatogonia, is not present. I have never been able to find more than ten separate chromosomes, and here, as in the male, the small number of chromosomes makes it easy to find a large number of dividing nuclei in which every chromosome is distinct (Pl. 12, figs. 4, 5). Since there is no Y, and since in the male, X is generally attached to an autosome, it is quite safe to interpret the ten chromosomes of the female as 10+XX, and the two X's attached to autosomes. The condition here is again comparable to that found in A. megalocephala, where Frolowa (1912) found that the two X-chromosomes are generally attached to autosomes in the female.

The Meiotic Phase.—The spermatogonial nuclei (Pl. 12, fig. 1) contain a very scanty chromatic reticulum and a large central nucleolus. This is apparently a plasmosome impregnated with chromatin, for it stains densely with iron haematoxylin, but in well-balanced methyl green and acid fuchsin preparations it takes up the fuchsin. In the early prophase of the spermatogonial mitoses this nucleolus loses its chromatic staining reaction even with iron haematoxylin, and becomes a typical plasmosome. This nucleolus is the only compact body in the resting spermatogonial nucleus, so unless they are somehow

incorporated with the plasmosome, it is clear that the sex chromosomes at this stage are in a diffused condition like the autosomes.

The earliest stages of the primary spermatocytes which are distinguishable from the spermatogonia are early leptotene stages—a rather later leptotene nucleus being shown in fig. 6. The large nucleolus is shown by its staining reaction to be a plasmosome, and is still the only compact body in the nucleus. The X-chromosome is therefore at this stage in the leptotene condition like the autosomes. The same presumably applies to the Y-chromosome, though this is too small to permit of definite statement.

The synizetic contraction, though unmistakable, is not very pronounced (Pl. 12, fig. 7).

The process of syndesis is difficult to follow in this animal. It begins about the stage shown in fig. 6, and is completed by the stage illustrated in fig. 7, which is a pachytene nucleus. About the stage of fig. 6 frequent duplicity and parallelism of threads can be observed, from which parasyndesis may be inferred. The direct evidence for this mode of syndesis is, however, certainly not strong in this species, but the indirect evidence is very convincing. Firstly, this mode of syndesis can be observed in Petauroides, and the general course of meiosis is so similar in the two genera that it is incredible that the mode of syndesis should not be the same. Secondly, as we shall see below, there is no doubt that the components of the definitive bivalents in Macropus are derived from the pachytene threads by the longitudinal splitting of these, and not by their doubling over. That being so, it follows that the mode of syndesis must have been by longitudinal fusion, unless one of the most fundamental hypotheses of modern cytology—that is to say the individuality of the chromosomes—is false.

In the early pachytene nuclei (Pl. 12, fig. 7), as in still earlier stages, the sex chromosomes are not visibly different from the other chromosomes. As the pachytene threads begin to contract, however, X soon becomes visible by reason of its much more rapid condensation, so that it soon comes to form

a compact rounded mass in sharp contradistinction to the still thread-like autosomes (Pl. 12, figs. 8–10). From its first appearance onwards it is attached to the end of one of the autosomes. At first it is not possible to identify the minute Y, but later, as the autosomes lose in staining capacity, Y becomes conspicuous by reason of its denser stain. At first it is distinct from X, but they soon fuse to form a bivalent (Pl. 12, fig. 9).

A large pale plasmosome makes its appearance at the time that the sex chromosomes are uniting and in close contact with them. The nature of this plasmosome, and its relation to the plasmosome of the earlier stages, is discussed below.

In the late pachytene stage (Pl. 12, figs. 9–10) the staining capacity of the autosomes becomes greatly diminished, and their outlines become somewhat blurred by the development of outgrowths and anastomoses between the different chromosomes. The compact XY bivalent is now very conspicuous, owing to the fact that the general decrease in staining capacity does not affect it nearly so much as the autosomes.

Fig. 11 represents the diplotene stage. This is perhaps chiefly interesting on account of the confidence with which its mode of derivation from the pachytene stage can be determined. In many late pachytene nuclei, such as about the stage shown in fig. 10, the five pachytene bands can be counted with ease and certainty, and one can follow step by step in great detail the conversion of each of these bands into one of the diplotene bivalents by the appearance and gradual widening of a longitudinal split down its middle. The three stages figured (Pl. 12, figs. 10, 11, 12) will, however, probably be enough to carry conviction that the gemini of the diplotene nucleus are derived from the pachytene bands in this way, and not by their doubling over as is required by the theory of telosyndesis.

Fig. 13 shows a stage in the contraction of the diplotene loops into the definitive bivalents. The nuclear membrane has by now disappeared. The great increase in bulk of the chromosomes which has taken place between the stages shown in figs. 11 or 12 and that shown in fig. 13 is remarkable. A

rough estimate of the relative volumes of the total chromatin content at these two stages made by means of plasticene models showed that the volume of the chromatin is more than twice as great in the later as in the earlier stage.

The XY bivalent is visible as before, attached to an autosome. It is now, however, seen to be attached to one limb only of the bivalent.

Figs. 14–16 (Pl. 13) represent metaphases of the first meiotic division, to show the relations of the sex chromosome. In fig. 14 they form a compact body attached to one end of one of the autosome bivalents. In fig. 15 they are similarly attached, but somewhat drawn out towards the equator of the spindle. At this stage no distinction between X and Y is visible, but as the metaphase progresses the two components begin to separate, as shown in figs. 16 A and B. The minute Y is very characteristically pulled out along the spindle-fibre at this stage.

Fifty metaphase I's were examined especially in respect to the mode of attachment of XY.

In fourteen cases it was attached as in fig. 14.

In twenty-five cases it was attached as in fig. 15.

In eleven cases it was apparently free, forming an independent bivalent. In many of these cases, however, it was probably attached as in the manner of fig. 15, but by a longer and finer thread. There is also little room for doubt that an attachment as in fig. 14 indicates an early metaphase, and that later the relations shown in fig. 15 are always assumed.

I have not been able to determine whether the autosome to which XY is attached is always the same one, but it appears probable that it is (note the distinctive shape of this chromosome in the two groups figured in fig. 13, and in figs. 14 and 15). The bivalent in question is, however, certainly always one of the larger ones.

The first meiotic division is the differential division for X and Y (Pl. 13, figs. 16, 17). During anaphase Y becomes still further pulled out along the line of the spindle-fibre, and presents in the late anaphase the characteristic appearance

illustrated in fig. 17. In Heidenhain preparations both X and Y are at this stage slightly paler than the autosomes.

Two kinds of secondary spermatocytes are therefore produced in the well-known manner, one with the X-chromosome and the other with the Y. There is a complete, and apparently prolonged, resting stage between the two divisions. The difference between the two kinds of secondary spermatocytes is conspicuous in the young nuclei, one member of each pair containing a dense chromatic body (presumably X) which is lacking in the sister nucleus or represented by a very much smaller speck (Pl. 13, fig. 18). A group of fully resting secondary spermatocytes, presenting the same dimorphism, is shown in fig. 19.

Fig. 20 shows a prophase for the second division in a secondary spermatocyte containing the larger chromatic body (X) which is seen attached to one of the chromosomes.

The expected two types of second division are easily found. An early, and rather irregular anaphase with the Y-chromosome is shown in fig. 21. Here Y has just divided. A later anaphase of the other type of second division is illustrated in fig. 22. Here we have apparently only five chromosomes present in each group; this must clearly be interpreted as a division in which X is present and fused, as usual, with an autosome.

It is noticeable that there is no trace in Macropus (nor in Petauroides) of the second pairing of chromosomes to give a quarter of the diploid number which has so often been described for the second division in birds and mammals.

Jordan (1911) described such a second pairing for Didelphys, but Painter (1922) found that it did not occur in his material.

B. Petauroides Volans.

Number of Chromosomes.—The determination of the number of chromosomes in this species presents more difficulty than in the case of Macropus, owing to their greater number. The number countable in the spermatogonial mitoses is generally twenty-two, forming typically a ring of twenty, with two smaller, slightly unequal ones, in the centre.

These are presumably X and Y, the latter being much larger than the corresponding element in Macropus, and being, indeed, but slightly smaller than X. I have, however, found some spermatogonial mitoses with apparently only twenty-one chromosomes, and yet containing this pair in the centre. I am therefore in doubt, from the spermatogonial mitoses, whether the number is 20+XY or 20+X. I have some quite unequivocal counts of polar views of the first meiotic metaphase, and some of these show eleven and some twelve separate elements. When twelve are present, one is always distinctly smaller than any of the others. Presumably, when the number is eleven, there are ten autosome bivalents and the XY bivalent. When the number is twelve, X and Y have dissociated. Side views of the meiotic metaphase (of which I have never found one that could be counted) show that one of the bivalents (? XY) commonly dissociates much in advance of the others. The conditions in the early pachytene nuclei also point to the presence of two sex chromosomes. It appears, therefore, to be fairly well established that the formula for the male Petauroides is 20+XY, or twenty-two in all. This corresponds with Painter's enumeration for Didelphys. It will also be noticed that the number of autosomes is double that of Macropus. None of my ovarian material proved suitable for chromosome counting.

The Meiotic Phase.—The spermatogonial nuclei of this animal differ from those of Macropus, in that the place of the fine reticulum of the latter genus is taken by a number of irregular blocks of chromatin, united by anastomoses. In the young spermatogonia these chromatic bodies are in approximately the diploid number, and from a study of the spermatogonial pro- and telophases it appears probable that these blocks are of the nature of 'prochromosomes', being the undiffused remains of the telophase chromosomes, and passing directly into the chromosomes of the following prophase. When the spermatogonia pass into a more profoundly resting stage the number of these bodies becomes more difficult to determine, owing to their becoming broken up and their

fragments scattered, till at last a stage is reached where the chromatin is finely distributed throughout the nucleus except for three or four masses representing the remains of the larger blocks which have escaped complete fragmentation. Very often, however, the number of the chromatic masses remains at approximately the diploid number throughout the whole interphase from the telophase to prophase.

The interstitial nuclei of the two genera present a similar difference, those of Macropus being finely reticular, and those of Petauroides containing about the diploid number of irregular chromatic masses.

The leptotene stage develops from a primary spermatocyte having the same structure as a spermatogonium, i.e. containing a number of massive chromatic bodies (Pl. 13, fig. 23). Each of these becomes the centre of a process of thread formation (Pl. 13, figs. 24, 25), to produce in the aggregate the leptotene nucleus (Pl. 13, fig. 26). This process resembles that by which the leptotene stage in certain insects is developed from a nucleus containing the diploid number of chromatic bodies (Wilson, 1912). In Petauroides, however, the evidence that these blocks represent each a single chromosome, though strong, is not complete.

The massive centres from which the thread-spinning starts persist for a long time, and indeed appear to form the basis of the synizetic knot. Synizesis is more pronounced in Petauroides than in Macropus—at least in my material (Pl. 13, fig. 27).

Parasyndesis occurs during the synizetic contraction (Pl. 13, figs. 27, 28), but not with the regularity observable in those animals in which the leptotene nucleus is orientated into a bouquet. In the nucleus shown in fig. 27 it is proceeding over one length of the thread, but appears to be already completed over the rest of the nucleus. That syndesis concerns, not the chromosomes as a whole but their constituent chromomeres, is beautifully shown in Petauroides (Pl. 13, figs. 27, 28). In this animal the chromomeres are unusually distinct and large at this, and some other, stages.

Figs. 27 and 28 show that, (1) the chromomeres in a single chromosome differ greatly in size, (2) the series of chromomeres in a pair of conjugating chromosomes closely corresponds, (3) conjugation takes place between the corresponding (homologous) chromomeres. It will be noticed also that the final union of chromomeres appears to be very intimate, all external trace of duplicity having disappeared in the most completely fused pairs.

Syndesis is followed by the usual pachytene stage (Pl. 14, figs. 29–33), during the early part of which two compact bodies, presumably X and Y, are conspicuous (Pl. 14, fig. 31). These soon unite into a single body, one or both of them often being pulled out into irregular shapes during the process (Pl. 14, fig. 32). The autosomes remain filamentar and suffer a temporary diminution of staining capacity. An important feature which is very conspicuous in Heidenhain preparations subjected to the right amount of extraction is that the stain is retained much more tenaciously by certain of the chromomeres than by others (Pl. 14, fig. 32). The general significance of chromomeres is discussed below.

As the chromosomes regain their staining powers towards the end of the pachytene stage, the chromomeres again become very conspicuous (Pl. 14, fig. 33), as they are also in the diplotene nucleus (Pl. 14, figs. 34, 35). It is interesting to compare the chromosomes shown in detail in fig. 35 with those in fig. 28, the latter representing the conjugation of the chromosomes, the former their separation. The correspondence between the chromomeres of homologous chromosomes is still evident in the diplotene stage, but now they are beginning to run together on the contracting chromosome, ultimately to give rise to the smooth chromosomes shown in fig. 36.

I have not been able to trace with certainty the movements of the sex chromosomes in the first meiotic division. In regard to the second division, all that can be said is that the number is clearly about ten or eleven, showing that there is no second numerical reduction.

The dimorphism of the secondary spermatocytes is not so

conspicuous as in Macropus, probably because the X and Y chromosomes are more nearly of the same size. Indeed, for a long time I thought there was no visible difference between the two types, but closer examination has shown that such a distinction exists. All secondary spermatocytes have a compact chromatic nucleolus, but in the case of young sister nuclei, whose relation to each other can still be seen by the persistent spindle remains, one of them constantly has a distinctly larger nucleolus than the other. Sometimes, as in the pair figured (Pl. 14, fig. 37), this is expressed by one of them being bilobed and the other single. In other cases it is merely a difference in size. The difference between the two classes of secondary spermatocytes is thus very small, but once recognized it is seen to be constant.

Discussion of some Special Problems raised by the foregoing Descriptions.

(1) Chromomeres.—Many cytologists maintain that chromomeres are purely artefacts, due to unequal contraction of the chromatic thread under the influence of the fixative, or else, in the case of smaller chromomeres, are mere optical effects of angles, &c., in the thread. At any rate, according to this view, they do not represent any real local differentiations of the substance of the chromosome.

The strength of the criticism that the chromomeres are artefacts depends much upon the exact meaning attached to that word. It is of little importance whether or not chromosomes which are beaded when fixed appear smooth in life (a very difficult observation in any case!) For the sake of argument it may be granted that the beading is produced by the action of the fixative. The important question is: Is the beading of such a nature that it could be produced mechanically by precipitation in and contraction of a homogeneous thread, or is it the expression of a pre-existing though perhaps invisible differentiation in the living chromosome? Doubtless so-called chromomeres have been described which might have been

produced by the action of the fixative on a homogeneous thread, but the view that the chromosomes are composed of differentiated chromomeres cannot be disposed of by demonstrating mistaken interpretation in individual cases. On the contrary, we have now a large accumulation of observations where the answer to the above question seems undoubtedly to be in the negative; observations, that is to say, which lead to the conclusion that whether the beads exist as such in life, or whether they are produced by the fixative at the moment of death, they must be expressions of local differentiations of the substance of the chromosome—and that is all, of course, that is required by the theory that connects the chromomeres with the linear arrangement of hereditary factors in a chromosome.

The reasons which seem to exclude the view that such chromomeres are produced mechanically, and so to speak, accidentally, on a homogeneous thread which is contracting unequally under the influence of unequal stresses in different parts are:

(1) The chromomeres in a single chromosome may differ very greatly as regard size (Pl. 13, figs. 27, 28).

(2) Had they been produced by unequal contraction of parts of a homogeneous thread, larger chromomeres would be separated from each other by longer intervals of connecting thread than those which separate the smaller chromomeres. A glance at fig. 28 shows that this rule does not hold.

(3) There is a close correspondence between the chromomeres of homologous chromosomes, both during syndesis and the diplotene stage.

(4) Wenrich (1916) has described the constant arrangement of the principal chromomeres on a given chromosome—a constancy which is maintained not only in all the nuclei (of the same stage) in a single animal, but even in different animals. Unless this observation be doubted, it supplies conclusive evidence that the chromomeres as seen in fixed nuclei correspond to definite local differentiations of the substance of the chromosome.

(5) It is now well known that the shape assumed by the long type of chromosome common in many forms of mitosis is

characteristic and constant for any given chromosome. This shape is commonly some form of V, with equal or unequal limbs. The point at which the chromosome bends to form the V—whether in the middle or towards one end—(which is also the spot to which the spindle-fibre is attached) varies from chromosome to chromosome, but is constant for any given chromosome. Similarly, the transverse constrictions which develop across the chromosomes of so many organisms are constant in position for a given chromosome. This constancy in position is proof of the existence of a constant differentiation, in a lengthwise direction, of the substance of the chromosome.

The tendency of certain chromosomes to develop transverse constrictions at spots constant for each particular chromosome is specially significant in estimating the value of the criticism that chromomeres are 'artefacts'. In Lepidosiren (Agar, 1913) the long somatic chromosomes usually show no trace of a transverse constriction. Each chromosome is a smooth curved rod or V of approximately uniform thickness. When the chromosomes become shorter and thicker, as happens regularly in the meiotic phase, and occasionally, from unknown reasons, in somatic tissues also, the transverse constrictions develop in a spot characteristic for each particular chromosome (which is also the point at which the apex of the V is situated, when the chromosome is in this shape).

This shortening and thickening of the chromosomes was produced in several plants by Sakamura (1920) by the action of various reagents, such as chloral hydrate and chloroform. These artificially shortened chromosomes showed well-marked and characteristically placed constrictions, though the position of these is barely indicated in normally fixed tissue. The constancy in position of these constrictions shows that they can only be called 'artefacts' in the sense that they make visible a pre-existing heterogeneity of the chromosome substance, which is concealed from view in the 'typical', well-fixed, and apparently uniform chromosome. Indeed, to deny that the chromomeres correspond to pre-existing local differentiations

of the chromosome substance on the ground that they only appear in tissues treated in a certain way, would be as illogical (granting that it is true) as to deny the distinction between a plasmosome and a chromatin nucleolus because the difference only becomes visible under the action of appropriate stains.

We conclude, therefore, that the chromomeres which appear in certain stages of mitosis in fixed tissues correspond to real local differentiations of the substance of the chromosome, though the actual shape which they assume (namely, bead-like swellings on a fine thread) may be assumed, or at least exaggerated, under the stress of the fixative.

(2) Crossing over.—Whether or not the phenomenon of crossing over occurs in mammals is still in doubt. Castle (1921) has described such a case in rabbits for the linked genes, English and non-English, and short-haired and Angora. Three individuals were tested, two males and a female, and crossing over was found in all of them. If this is established, it will show that the phenomenon in mammals is not quite comparable to that in Drosophila and Bombyx, where it occurs only in the sex which is homozygous for the sex chromosomes. By analogy with these, crossing over is to be expected in the female mammal alone. Considering the cytological evidence only, it would certainly seem that the conditions supposed to be necessary for crossing over are provided in the male diplotene nuclei of both these genera.

This is specially clear in Petauroides, because of the chromomeres. As fig. 27 shows, fusion of chromomeres in syndesis is intimate. Indeed, no sign of duplicity may remain. In fig. 35 (diplotene stage) the intertwined chromosomes are still held together at certain of the crossing places by unsplit chromomeres, and in view of their intimate union it is not difficult to imagine that when they finally do separate they may do so in such a way that the portions of the two chromosomes on either side of the point of union have been interchanged.

(3) The Relation between the Sex Chromosomes and the Plasmosomes in the Meiotic Phase.—The

condition of the plasmosome in Macropus has already been described. In Petauroides a plasmosome can occasionally be seen in the primary spermatocytes, but usually none can be identified with certainty—probably because, as in Macropus, this plasmosome is partly chromatic in the resting nucleus, and therefore does not stand out clearly from the other chromatic bodies. In the leptotene, synizetic, and early pachytene nuclei of Petauroides, a plasmosome can sometimes be identified, but is usually concealed among the dense tangle of chromatic threads. In the later pachytene stages, two plasmosomes are plainly visible (Pl. 14, fig. 32). One is considerably darker than the other, and has no relation to the sex chromosomes. This one I take to be the plasmosome of the earlier stages. The other plasmosome is in close relation to the sex chromosomes, and indeed appears to be formed out of their substance. It makes its appearance as a large pale body at the time that the sex chromosomes are uniting into a bivalent, and at first is an elongated structure closely attached to the bivalent. In some cases its shape and relations suggest that it is the persistent part of the bivalent from which the chromatin has flowed away into the rounded mass which forms the condensed sex bivalents : this appearance is strengthened by the fact that sometimes rounded granules or drops of chromatin are left embedded in the plasmosome. In other cases it is pear-shaped and is attached by its neck to the bivalent, irresistibly suggesting that it has been squeezed out of the contracting chromosomes like a viscid fluid from a narrow aperture. These appearances are illustrated in figs. 32 and 38. In later stages this plasmosome becomes approximately spherical and often becomes detached from the sex bivalent, though always lying close to it.

For a time the two plasmosomes—one presumably the remains of the pre-leptotene nucleolus, and the other apparently formed out of material (plastin or linin ?) derived from the sex chromosomes—coexist, the former being the first to disappear.

In Macropus the larger pale plasmosome which appears in close connexion with the uniting sex chromosomes has also the

appearance of being formed out of their substance, though no figures were discovered quite so striking as those illustrated for Petauroides.

(4) Chromatoid Bodies.—In Petauroides one or two bodies staining densely with iron haematoxylin appear suddenly in the cytoplasm in the pachytene stage. Their origin and fate have not been determined, but they seem to be distributed capriciously at cell-division. In fig. 37 they have all passed to one daughter cell (that containing the sex chromosome), but this mode of allocation is not invariable. In Macropus chromatoid bodies are either absent or inconspicuous.

Summary.

Macropus ualabatus has twelve chromosomes, namely 10 + XY in the male and 10 + XX in the female.

In Petauroides the number is almost certainly twenty-two, the male being of the formula 20 + XY. No female counts were obtained for this animal.

In the male Macropus X is generally attached to one of the autosomes in spermatogonial mitoses. Y, which is exceedingly minute, is free. During the pachytene stage, while the autosomes are still elongated, X and Y condense into a bivalent. In the first meiotic division this bivalent is attached to an autosome.

As a result of the first meiotic division the usual two classes of secondary spermatocytes are formed one with X and the other with Y. In the second meiotic division, those with X show only five separate chromosomes, showing that X, as usual, is fused with an autosome. The other class of second divisions shows five autosomes and the minute Y.

In the female Macropus the sex chromosomes were never found free from the autosomes in the ovarian follicle cells, which therefore show only ten separate chromosomes.

In Petauroides the sex chromosomes cannot be distinguished with certainty from the autosomes. An unequal pair of small chromosomes usually situated in the centre of the

spermatogonial metaphase plates probably, however, are X and Y. Early pachytene nuclei show two compact bodies which unite into one, presumably the sex bivalent.

The second reduction of the chromosome number to one-quarter of the diploid total in the second meiotic division, which has been described for several species of birds and mammals, does not take place either in Macropus or Petauroides.

Chromomeres are very prominent in Petauroides in the zygotene and diplotene stages.

Probably in Macropus, and more convincingly in Petauroides, the cytological conditions to permit of 'crossing over' are present in the male.

The plasmosome which appears in the pachytene stage is probably formed from the plastin or linin basis of the contracting sex chromosomes.

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EXPLANATION OF PLATES 12, 13, AND 14.

The scale given on Pl. 12 applies to all the figures except 19 and 38, which are on a smaller scale.

Lettering.

P, pre-pachytene plasmosome. Px, plasmosome which appears at the time that the sex chromosomes are uniting. X, Y, the sex chromosomes.

Figs. 1-22, Macropus ualabatus; Figs. 23-38, Petauroides volans.

Plate 12.

Fig. 1.—Resting spermatogonium.

Fig. 2.—Metaphase, spermatogonial mitosis, eleven chromosomes.

Fig. 3.—Metaphase, spermatogonial mitosis, twelve chromosomes.

Figs. 4, 5.—Metaphase, ovarian follicle cells, ten chromosomes.

Fig. 6.—Late leptotene nucleus.

Fig. 7.—Synizesis.

Fig. 8.—Early pachytene nucleus showing condensation of X.

Fig. 9.—Later stage, showing pairing of X and Y.

Fig. 10.—Late pachytene nucleus.

Fig. 11.—Early diplotene nucleus.

Fig. 12.—The chromosomes of an early diplotene nucleus shown separately.

Fig. 13.—Contracting bivalents in two adjacent cells.

Plate 13.

Figs. 14, 15.—Two metaphases of the first meiotic division, to show modes of attachment of XY to an autosome.

Figs. 16 A, B.—Autosomes, with attached XY bivalent, from more advanced metaphases, to show separation of X and Y.

Fig. 17.—Anaphase of first division.

Fig. 18.—Pair of young secondary spermatocytes, still connected by the spindle remains, one with large compact chromatic body, the other without.

Fig. 19.—A group of secondary spermatocyte nuclei to show the dimorphism. Half with large and half with small chromatic body (presumably X and Y).

Fig. 20.—Early prophase of a second division with the X-chromosome.

Fig. 21.—Anaphase of a second division with the Y-chromosome. A small cyphlasmic inclusion is shown at the bottom right-hand corner.

Fig. 22.—Anaphase of a second division with the X-chromosome (indistinguishably fused with an autosome). To avoid overlapping the two groups have been slightly shifted laterally in drawing.

Fig. 23.—Resting primary spermatocyte.

Figs. 24, 25.—Fragments of developing leptotene nuclei to show conversion of the massive blocks of the resting spermatocyte into the leptotene threads.

Fig. 26.—Leptotene nucleus.

Fig. 27.—Synizesis and syndesis.

Fig. 28.—Three short lengths of conjugating chromosomes from three different zygotene nuclei.

PLATE 14.

Fig. 29.—Syndesis complete.

Fig. 30.—Early pachytene nucleus, synizesis loosening out.

Fig. 31.—Pachytene stage, with two compact bodies, presumably X and Y.

Fig. 32.—Later stage. X and Y, one of them greatly pulled out, uniting into a bivalent.

Fig. 33.—Late pachytene nucleus showing evidence of commencement of diplotene stage. Chromatoid body in the cytoplasm.

Fig. 34.—Early diplotene nucleus. Chromatoid body in cytoplasm.

Fig. 35.—Three bivalents from an early diplotene nucleus.

Fig. 36.—Late diplotene nucleus, chromatoids in cytoplasm.

Fig. 37.—A pair of young secondary spermatocytes, still connected by the spindle remains. Note larger and bilobed chromatic body in the upper nucleus. Chromatoids in the cytoplasm of one cell.

Fig. 38.—Outline drawings of four nuclei, about the stage of fig. 32, to show relations between the sex chromosomes and the plasmosome.